Remarks

The Office Action mailed September 30, 2002, has been received and reviewed. Claims 1-29 and 34 having been canceled and claims 30-33, 35-37 and 43-45 having been amended, the pending claims are claims 30-33 and 35-66. Of these, claims 35, 36 and 46-66 have been withdrawn from consideration by the Examiner as being drawn to non-elected inventions. Claims 30-33 and 37-45 are therefore presently under examination.

Support for the recitation of a pharmaceutical composition in claim 37, as amended, is found, for example, at page 8, lines 6-7. Support for the recitation of an "immunogenic" subunit in amended claims 37 and 43 is found in the specification at, for example, page 49, lines 3-17. New claim 67, drawn to an immunogenic composition that generates an antibody response when administered to a subject, is supported for example by the specification at page 12, lines 7-8.

Informalities in the Specification

The Examiner objected to the specification because the first paragraph does not accurately reflect the issued status of the parent application, U.S. Patent Application Serial No. 09/282,352. As suggested by the Examiner, the first paragraph of the specification has been amended to indicate that this application issued as U.S. Patent No. 6,187,321, on February 13, 2001.

The Examiner also objected to the specification insofar as it does not reflect the proprietary nature of certain trademarks. The specification has been amended at various locations to indicate that materials are referred to by their tradenames, and to correct obvious typographical errors.

The Examiner also objected to the specification because it includes embedded active hyperlinks and/or browser executable code. The specification has been amended such that it does not include active hyperlinks and/or browser executable code.

The Examiner also objected to the specification because the address of the American Type Culture Collection has changed. The specification has been amended to recite the new address of the ATCC.

It is respectfully submitted that the objections to the specification have been obviated as a result of the amendments to the specification.

Double Patenting Rejection

Claims 37-44 were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-5 of U.S. Patent No. 6,087,128. Claims 30-34, 43 and 44 were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 17 of U.S. Patent No. 6,087,128.

This rejection is respectfully traversed. Claim 34 has been canceled, without prejudice. Upon an indication of otherwise allowable subject matter and in the event this rejection is maintained as to the remaining claims, Applicants will provide an appropriate response to the double patenting rejection. Applicants acknowledge the Examiner's statement that a timely filed Terminal Disclaimer may be used to overcome this rejection.

Rejection under 35 U.S.C. §101

Claim 37 is rejected under 35 U.S.C. §101 as being directed to non-statutory subject matter. The claim encompasses an immunogenic composition comprising a non-isolated nucleic acid molecule encoding, *inter alia*, an avian *E. coli* Iss polypeptide. The Examiner alleges that the claim reads on products of nature.

This rejection is traversed. However, in order to expedite examination of the instant application, claim 37 is amended to recite a composition that further includes a pharmaceutically acceptable carrier. It is respectfully submitted that the amendment obviates the rejection under 35 U.S.C. §101. Reconsideration and withdrawal of the rejection is respectfully requested.

Rejection under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 30-34 and 37-45 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

Claim 30 was rejected as being vague in the recitation of "73 to 309 of SEQ ID NO:22 without reciting that SEQ ID NO:22 is a nucleotide sequence. Claim 30 has been amended to recite --73 to 309 of the nucleotide sequence SEQ ID NO:22-- in accordance with the suggestion of the Examiner. Claims 31, 33, 35, 36, 43, 44 and 45 have been analogously amended. Claim 34 has been canceled, without prejudice.

Claim 32 was rejected for lacking proper antecedent basis, and has been amended to recite nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

Claim 33 was rejected as being vague in the recitation of "to a least one." This was at typographical error, and the claim has been amended to recite "to *at* least one".

Claim 34 was rejected as being vague and indefinite in the recitation of "under stringent conditions." Claim 34 has been canceled, rendering the rejection moot.

Claims 37 and 43 were rejected as being vague and indefinite in the recitation of "subunit" of an avian *E. coli* Iss polypeptide. Claims 37 and 43 (as amended) recite an "immunogenic subunit" of avian *E. coli* Iss polypeptide. The Examiner's attention is directed the specification at page 49, lines 3-17, wherein it is stated:

> An *immunogenic subunit* of an *E. coli* Iss polypeptide is a subunit that elicits an immune response in a subject to which it is administered. An immune response includes either or both of a cellular immune response or production of antibodies, and can include activation of the subject's B cells, T cells, helper T cells or other cells of the subject's immune system. For example, an immune response is evidenced by a detectable anti-*E. coli* Iss polypeptide antibody level in the subject using methods of antibody detection known to the art.
>
> *Immunogenicity* of an *E. coli* Iss polypeptide or subunit can be determined, for example, by administering the adjuvanted candidate subunit to the subject, then observing of the associated immune response by analyzing anti-*E. coli* Iss subunit antibody titers in serum. An immunogenic subunit preferably contains more than 7 amino acids, more preferably at least about 10 amino acids, most preferably at least about 20 amino acids. When an immunogenic subunit of

> an *E. coli* Iss polypeptide or subunit is identified, the nucleic acid sequence encoding the subunit can be inserted into an appropriate expression vector, for instance pVAX1 (emphasis added).

It is respectfully submitted that the term "immunogenic subunit" is clear in view of the teachings of the specification. As claim 37 is not vague or indefinite, it is respectfully submitted that the claims that depend from claim 37 (namely, claims 38-45) are not indefinite.

Claim 42 was rejected as being vague and indefinite in the recitation of "immunostimulatory sequence" in that it is unclear whether this is a nucleotide sequence or an amino acid sequence. Claim 42 is amended to recite an "immunostimulatory nucleotide sequence." In this regard, the Examiner's attention is drawn to page 49, lines 20-25:

> In addition, the vector construct can contain immunostimulatory sequences that stimulate the animal's immune system. Examples of immunostimulatory sequences include, for instance, sequences with CpG motifs, two 5' purines, an unmethylated CpG dinucleotide, or two 3' pyrimidines (see, for instance, Lowie et al., DNA Vaccines Methods and Protocols, Humana Press, Totowa, NJ (2000)).

Claim 43 was rejected as being vague and indefinite in the recitation of "fragment thereof." Further, claim 43 was rejected as improperly broadening in scope in view of the recitation of "immunogenic fragment" in claim 38, from which it depends. Claim 43 has been amended to recite an "immunogenic fragment" thereby obviating these rejections.

Reconsideration and withdrawal of the rejection of claims 30-34 and 37-45 under 35 U.S.C.§112, second paragraph, is respectfully requested.

Rejection under 35 U.S.C. §102

The Examiner rejected claims 30 and 34 under 35 U.S.C. §102(a) as being anticipated by GenEmbl Accession AF042279, GI:5305230 (Horne, Nolan, Giddings and Pfaff). This rejection is respectfully traversed.

Claim 34 has been canceled, without prejudice, rendering the rejection moot as to that claim.

Co-authorship of a reference, while sufficient to support a *prima facie* case under 35 U.S.C. §102(a), does not establish co-inventorship. Applicants Nolan and Horne submit that they alone invented the subject matter disclosed in the cited reference, despite the fact that the authorship of the reference includes two non-Applicants, Giddings and Pfaff. To support their contention, Applicants submit herewith a Declaration under 37 C.F.R. §1.132 setting forth the relevant facts regarding inventorship. It is respectfully submitted that the cited reference is therefore not the work "of another" and is not prior art under 35 U.S.C. §102(a). *In re Katz*, 215 U.S.P.Q. 14, 18 (CCPA 1982); MPEP §716.10.

Reconsideration and withdrawal of the rejection of claim 30 and 34 under 35 U.S.C. §102(a) as being anticipated by GenEmbl Accession AF042279, GI:5305230 (Horne, Nolan, Giddings and Pfaff) is respectfully requested.

The Examiner rejected claims 34, 37-40 and 43 under 35 U.S.C. §102(b) as being anticipated by Barondess et al. (*Nature 344*:871-874 (1990)) or Chuba et al. (*Mol. Gen. Genet. 216*:287-292 (1989)). This rejection is respectfully traversed.

As noted above, claim 34 has been canceled, without prejudice, rendering the rejection moot with respect to that claim. Claim 37, from which claims 38-40 and 43 depend, has been amended to read as follows:

37. An immunogenic composition comprising:
a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an avian *E. coli* Iss polypeptide or an immunogenic fragment or immunogenic subunit thereof; and
a pharmaceutically acceptable carrier.

Chuba et al. teach the cloning and sequence of an *iss* gene from conjugative plasmid ColV2-K94. This study reports increased serum survival and surface exclusion characteristics of *E. coli* K12 cells that carry an *iss* gene fragment.

Barondess et al. teach a lambda bacteriophage *bor* sequence that is homologous with the ColV2-K94 *iss* locus. Expression of *bor* significantly increases the survival of the *E. coli* host cell in animal serum. This property is a well known bacterial virulence determinant. Barondess et al. further state that lysogeny by lambda phage may generally have a role in bacterial survival in animal hosts and perhaps in pathogenesis.

In the Examiner's view, it is inherent in the teachings of Chuba et al. and Barondess et al. that the nucleic acid taught therein serves intrinsically as an immunogenic composition. The Examiner asserts that the immunogenic function is viewed as an inherent property of the prior art nucleic acid molecule which meets the structural elements of the claimed product.

Applicants disagree. The cited documents evaluate the ability of *bor* or *iss* to increase survival of *E. coli* K12 in guinea pig serum. At page 873, second column, Barondess at al. teach that "this is the first example known to [the authors] describing a phage-encoded function that could provide direct protection from host immune defences" and further that "[r]esistance to serum-complement killing is one of the most extensively studied bacterial virulence determinants." The Examiner has not provided any evidence as to why increased virulence is necessarily and inevitably equated with therapeutic immunogenicity, in order to support reliance on a theory of inherency under 35 U.S.C. §102(b). To the contrary, increased virulence (survival time) would imply anti-therapeutic effects attributable to the genes or gene products.

"In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art" (emphasis in original). M.P.E.P §2112. "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic" (emphasis in original). M.P.E.P §2112. "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however,

may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' " M.P.E.P §2112. Applicants maintain that the doctrine of inherency is not available to supplement the demonstrated deficiencies of the cited documents.

Furthermore, claim 37 has been amended to recite a pharmaceutically acceptable carrier. Pharmaceutical compositions are neither taught nor suggested in the cited references, suggesting that any potential use of *bor* and *iss* (or fragments or subunits thereof) as therapeutic immunogens, for example as in a DNA vaccine, was unrecognized in the art. Likewise these references do not teach or suggest immunizing subjects against pathogenic *E. coli* by treating them with a pharmaceutical compositon that contains all or part of an *iss* gene.

Reconsideration and withdrawal of the rejection of claims 34, 37-40 and 43 under 35 U.S.C. §102(b) as being anticipated by Barondess et al. (*Nature 344*:871-874 (1990)) or Chuba et al. (*Mol. Gen. Genet. 216*:287-292 (1989)) is respectfully requested.

Summary

It is respectfully submitted that the pending claims 30-33 and 35-66 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for
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CERTIFICATE UNDER 37 CFR §1.8:
The undersigned hereby certifies that this paper is being transmitted by facsimile in accordance with 37 CFR §1.6(d) to the Patent and Trademark Office, addressed to Assistant Commissioner for Patents, Washington, D.C. 20231, on this **30th** day of **JANUARY, 2003**, at 2:09 pm (Central Time).

By:
Name: SAM HER

APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS INCLUDING NOTATIONS TO INDICATE CHANGES MADE
Serial No.: 09/738,599
Confirmation No.: 1240
Docket No.: 255.0001 0122

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Specification

Please replace the paragraph beginning at **page 1, line 5**, with the following rewritten paragraph.

This application is a continuation-in-part of U.S. Patent Application Serial No. 09/282,352, filed March 31, 1999, now U.S. Patent No. 6,187,321, issued February 13, 2001, which is a divisional of U.S. Patent Application Serial No. 09/023,221, filed February 12, 1998, now U.S. Patent 6,087,128, issued July 11, 2000, each of which is incorporated by reference herein.

Please replace the paragraph beginning at **page 10, line 28**, with the following rewritten paragraph.

The percent sequence identity between a "variant" Iss polypeptide and a full-length Iss polypeptide, *e.g.*, SEQ ID NO:2, is generally determined by aligning the residues of the two amino acid sequences (i.e., a candidate amino acid sequence and the amino acid sequence of SEQ ID NO:2) to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. A candidate amino acid sequence is the amino acid sequence being compared to an amino acid sequence present in SEQ ID NO:2. A candidate amino acid sequence can be isolated from an avian *E. coli*, or can be produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, two amino

acid sequences are compared using the Blastp program, version 2.1.2, of the BLAST 2 search algorithm, as described by Tatusova, et al. (*FEMS Microbiol Lett* 1999, 174:247-250), and available from the worldwide web at [www.]ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, percent identity is referred to as "identities."

Please replace the paragraph beginning at **page 12, line 17**, with the following rewritten paragraph.

An isolated "variant" nucleic acid sequence of the present invention is a nucleic acid sequence that has at least 87%, preferably at least about 90%, and most preferably at least about 95%, but less than 100%, nucleic acid sequence identity or homology to a the nucleotide sequence of the corresponding wild type nucleic acid molecule, e.g., a DNA sequence comprising SEQ ID NO:22. However, a variant nucleic acid molecule of the invention may include nucleotide bases not present in the corresponding wild type nucleic acid molecule, as well as internal deletions relative to the corresponding wild type nucleic acid molecule. As used herein a nucleic acid "subunit" is a biologically active portion or region of a full-length *iss* nucleic acid sequence, *e.g.*, SEQ ID NO:22, or a portion or region of an *iss* nucleic acid sequence that encodes a biologically active subunit of an Iss polypeptide. The percent identity between a "variant" nucleic acid sequence of the present invention and a wild type nucleic acid molecule, e.g., SEQ ID NO:22, is determined by aligning the residues of the two polynucleotides (i.e., the candidate nucleotide sequence and the nucleotide sequence of the coding region of SEQ ID NO:22) to optimize the number of identical nucleotides along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of shared nucleotides, although the nucleotides in each sequence must nonetheless

remain in their proper order. A candidate nucleotide sequence is the nucleotide sequence being compared to SEQ ID NO:22. A candidate nucleotide sequence can be isolated from an *E. coli*, preferably an *E. coli* obtained from a bird, or can be produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, two nucleotide sequences are compared using the Blastn program, version 2.1.2, of the BLAST 2 search algorithm, as described by Tatusova, et al. (*FEMS Microbiol Lett* 1999, 174:247-250), and available from the worldwide web at [www.]ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including reward for match = 1, penalty for mismatch = -2, open gap penalty = 5, extension gap penalty = 2, gap x_dropoff = 50, expect = 10, wordsize = 11, and filter on. In the comparison of two nucleotide sequences using the BLAST search algorithm, percent identity.

Please replace the paragraph beginning at **page 21, line 3**, with the following rewritten paragraph.

When the avian *E. coli iss* sequence was examined with GENE INSPECTOR [Gene Inspector] software (Textco, Inc., West Lebanon, New Hampshire), it was found that the avian *E. coli* Iss protein is predicted to have an isoelectric point of approximately 8.47, and at pH 7, is expected to have a net charge of +2.05. An Iss polypeptide is predicted to be approximately a 10-11 kD protein containing 102 amino acids that is resistant to acid hydrolysis. Additionally, based on the Iss polypeptide's predicted folding characteristics and hydropathy plots, Iss is likely to have a number of accessible sites, for example, sites not buried in the bacterial membrane, that are antigenic.

Please replace the paragraph beginning at **page 35, line 5**, with the following rewritten paragraph.

The Merrifield synthesis method commences from the carboxy-terminal end of the peptide using an alpha-amino protected amino acid. Fluorenylmethyloxy-carbonyl (Fmoc) or t-butyloxycarbonyl (Boc) protective groups can be used for all amino groups even though other protective groups are suitable, and the first protected amino acids can be esterified to chloromethylated polystyrene resin supports. The polystyrene resin support is preferably a copolymer of styrene with about 0.5 to 2% divinyl benzene as a cross-linking agent which causes the polystyrene polymer to be insoluble in certain organic solvents. See Carpino *et al.*, J. Org. Chem., 37:3404 (1972); Meinenhofer, Int. J. Peat. Pro. Res., 11:246 (1978); and Merrifield, J. Am. Chem. Soc., 85:2149 (1963). The immobilized peptide is then N-deprotected and other amino acids having protected amino groups are added in a stepwise manner to the immobilized peptide. At the end of the procedure, the final peptide is cleaved from the resin, and any remaining protecting groups are removed by treatment under acidic conditions, for example, with a mixture of hydrobromic acid and trifluoroacetic acid. Alternatively, the cleavage from the resin may be effected under basic conditions, for example, with triethylamine, where the protecting groups are then removed under acidic conditions. The cleaved peptide is isolated and purified by means well known in the art, for example, by lyophilization followed by either exclusion or partition chromatography on polysaccharide gel media such as SEPHADEX [Sephadex] G-25, or countercurrent distribution. The composition of the final polypeptide may be confirmed by amino acid analysis after degradation of the polypeptide by standard means.

Please replace the paragraph beginning at **page 35, line 27**, with the following rewritten paragraph.

The synthesis may use manual techniques or be completely automated. For example, an APPLIED BIOSYSTEMS 431A PEPTIDE SYNTHESIZER [Applied BioSystems 431A Peptide Synthesizer] (Foster City, Calif.) or a BIOSEARCH [Bioscarch] SAM II automatic peptide synthesizer (Biosearch, Inc., San Rafael, Calif.) can be employed following the directions provided in the instruction manual and reagents supplied by the manufacturer. Disulfide bonds between Cys residues can be introduced by mild oxidation of the linear peptide by KCN as taught in U.S. Pat. No. 4,757,048 at column 20.

Please replace the paragraph beginning at **page 41, line 24**, with the following rewritten paragraph.

Responding mice are given a final booster consisting of about 5-100 μg, preferably 25-50 μg of antigen, preferably without adjuvant, administered intravenously. Three to five days after final boosting, spleens and sera are harvested from all responding mice, and sera is retained for use in later screening procedures. Spleen cells are harvested by perfusion of the spleen with a syringe. Spleen cells are collected, washed, counted and the viability determined via a viability assay. Spleen and SP2/0 myeloma cells (ATCC, [Rockville, MD] 10801 University Boulevard, Manassas, VA 20110-2209) that have been screened for HAT sensitivity and absence of bacterial contamination are combined, the suspension pelleted by centrifugation, and the cells fused using polyethylene glycol solution. The "fused" cells are resuspended in HT medium (RPMI supplemented with 20 % fetal bovine serum (FBS), 100 units of penicillin per ml, 0.1 mg of streptomycin per ml, 100 μM hypoxanthine, 16 μM thymidine, 50 μM 2-mercaptoethanol and 30 % myeloma-conditioned medium) and distributed into the wells of microtiter plates. Following overnight incubation at 37°C in 5% CO_2, HAT selection medium (HT plus 4 μM

aminopterin) is added to each well and the cells fed according to accepted procedures known in the art. In approximately 10 days, medium from wells containing visible cell growth are screened for specific antibody production by ELISA. Only wells containing hybridomas making antibody with specificity to Iss, or GST-Iss, are retained. The ELISA is performed as described above, except that the primary antibody added is contained in the hybridoma supernatants. Appropriate controls are included in each step.

Please replace the paragraph beginning at **page 44, line 22**, with the following rewritten paragraph.

Examples of adjuvants or carriers that may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glyccro-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL + TDM + CWS) in [a] an emulsion containing 2% squalene/[Tween] TWEEN 80 [tradename] emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against an immunogenic polypeptide containing an Iss antigenic sequence resulting from administration of the polypeptide (or a nucleic acid encoding the polypeptide) in immunogenic compositions or vaccines that are also comprised of the various adjuvants.

Please replace the paragraph beginning at **page 56, line 27**, with the following rewritten paragraph.

Polypeptides prepared from the pGEX-6P-3 expression vector yielded a glutathione S-transferase-Iss ("GST-Iss") fusion polypeptide product that is readily purified from the bacterial lysates by affinity chromatography under mild, non-denaturing conditions. Specifically, a bacterial sonicate is applied to a column of [glutathione sepharose] GLUTATHIONE SEPHAROSE 4B [Pharmacia Biotech Inc., Piscataway, N.J.] at 4° C and washed three times with 10 bed volumes of 1X PBS. Glutathione elution buffer (10 mM reduced glutathione in 50 mM Tris-HCl (pH 8.0)) is added to the column and incubated at room temperature (about 22-25° C) for 10 minutes, and the fusion protein is eluted. Eluates recovered from the column contain the fusion protein.

Please replace the paragraph beginning at **page 57, line 7**, with the following rewritten paragraph.

Alternatively, after expression of GST-Iss in *E. coli*, the bacteria are lysed by sonication, and the insoluble material is pelleted and removed, and the supernatant passed through a slurry of [Glutathione Sepharose] GLUTATHIONE SEPHAROSE 4B (Pharmacia Biotech Inc., Piscataway, N.J.) to permit binding of the GST-Iss fusion polypeptide to the Sepharose beads. To remove GST-Iss from other cellular proteins, the "bead-bound" fusion polypeptide is pelleted by centrifugation and washed with 1X PBS. The desired product is eluted from the Sepharose by the addition of reduced glutathione (Pharmacia Biotech Inc., Piscataway, NJ). Upon [] removal from the [Sepharose] SEPHAROSE beads, the purified GST-Iss fusion product is cleaved into Iss and GST by a site-specific protease, such as [PreScission Protease] PRECISSION PROTEASE (Pharmacia Biotech Inc., Piscataway, NJ), and the remaining GST is

separated from Iss by the same procedure used to purify the GST-Iss fusion polypeptide. The resulting polypeptide products are then analyzed by SDS-PAGE.

Please replace the paragraph beginning at **page 60, line 8**, with the following rewritten paragraph.

Restriction enzyme digest plasmid fragments and amplification products were separated by horizontal gel electrophoresis. Amplified fragments were identified by size, excised from the agarose and purified using GENECLEAN™ (Bio101, La Jolla, Calif.) or the WIZARD PCR CLEAN-UP SYSTEM [Wizard PCR Clean-Up System] (Promega, Madison, WI). The identities of the amplicons were further confirmed by sequencing according to the procedures described below. To prepare probes, isolated fragments were labeled using a non-radioactive, random-primed DNA labeling kit (GENIUS I [Genius I] Labeling and Detection Kit, Boehringer Mannheim, Indianapolis, Ind.).

Please replace the paragraph beginning at **page 61, line 1**, with the following rewritten paragraph.

The first series of cycles were the labeling reactions, wherein an IRD40-dATP was added to the extended primer sequence. The second series of cycles was the termination reaction for which 4 µl of the labeling reaction was added to 2 µl of each ddNTP termination mix before cycling. Unincorporated label was removed from the products by ethanol precipitation according to manufacturer's directions. Samples were separated on 4.0 % LONG RANGER [Long Ranger] acrylamide gels (FMC, Rockland, ME) and analyzed via a LI-COR 4000 LR automated sequencer.

Please replace the paragraph beginning at **page 61, line 18**, with the following rewritten paragraph.

The prepared *E. coli* DNA was added to a master mix consisting of 47.5 - 53.5 µl ddH_20, 10.0 µl of 10X PCR Buffer II (Perkin Elmer or Promega, Madison, WI.), 0.5 µl of [Amplitaq] AMPLITAQ DNA polymerase (Perkin Elmer), 1.0 µl of 0.1 mM of each appropriate primer and 4.0 µl or 8.0 µl of 25 mM $MgCl_2$.

Please replace the paragraph beginning at **page 61, line 22**, with the following rewritten paragraph.

The amplification cycles were as previously described except that the annealing temperature was 49 °C or 51.8 °C depending on the primer pair used. Amplified fragments for ligation into the expression vector pGEX-6P-3, were digested with *Bam*HI and *Eco*RI to produce sticky ends for the ligation process. Amplified fragments for ligation into pGEM-T vector have "A" overhangs left by Taq polymerase. Restriction enzyme digest plasmid fragments and amplification products were separated by horizontal gel electrophoresis. Amplified fragments were identified by size, excised from the agarose and purified using the WIZARD PCR CLEAN-UP SYSTEM [Wizard PCR Clean-Up System] (Promega, Madison, WI). T7 DNA ligase was used to ligate amplification fragments into the vectors. The identities of the amplicons were further confirmed by sequencing according to the procedures described below.

Please replace the paragraph beginning at **page 67, line 22**, with the following rewritten paragraph.

To prepare samples for analysis by flow cytometry, bacteria are grown in Brain Heart Infusion (BHI) broth for 18 hours at 37° C. Bacteria are then washed and resuspended in buffer.

Monoclonal antibodies specific for Iss are added to each bacterial suspension and allowed to incubate for 30 minutes. Suspensions are pelleted, washed thoroughly, and incubated with FITC-labeled, goat anti-mouse Ig conjugated antibody for 30 minutes at 0° C. After incubation, suspensions are pelleted, washed thoroughly, fixed with paraformaldehyde, and analyzed by flow cytometry using a FALSCALIBUR [FACSCalibur] (Becton Dickinson, San Jose, CA) (Otten *et al.*, Flow Cytometry Analysis Using the Becton Dickinson FACScan, John Wiley & Sons, New York, pp. 5.4.1-5.4.19 (1992)).

Please replace the paragraph beginning at **page 68, line 8**, with the following rewritten paragraph.

Cloning of *iss*. The primers for amplification of *iss* were designed using the PRIMERSELECT [PrimerSelect] program of LASERGENE [LaserGene] (DNAStar, Madison, WI) based on the DNA sequence in Horne et al. (*Avian Dis.*, 44: 179-184 (2000); GenBank Acc. No. AF042279; SEQ ID NO:1). To facilitate cloning, these primers were modified to add restriction sites (Bam*HI* on the upper primer and Eco*RI* on the lower primer at the 5' ends). The modified nucleotides are present in the primers as non-italicized text, and the italicized nucleotides hybridize with nucleotides present in SEQ ID NO:1 or the complement thereto. The primers were *iss* pVAX1 Upper, 5'AGTGGGGATCC*TAACAATGCAGGATAATAAGATGA* (SEQ ID NO:23); and *iss* pVAX1 Lower, 5'ATGCGGAATTC*TGTAGGGAGCCCAGAAGTA* (SEQ ID NO:24).

Please replace the paragraph beginning at **page 68, line 19**, with the following rewritten paragraph.

Based on the unmodified primers, amplification conditions recommended by PRIMERSELECT [PrimerSelect] were determined, and these were used to amplify *iss* from the

virulent avian *E. coli*. DNA template from the *E. coli* isolate used in the amplification was prepared as follows. A colony was lysed in a solution containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 50 µg/ml proteinase K by incubation at 55°C for 10 minutes followed by incubation at 80°C for 10 minutes. After dilution with a 2x volume of ddH_2O, cellular debris were removed by a brief centrifugation (30 seconds at 10,000 x g), and the supernatant containing the DNA was transferred to a fresh tube. Five microliters of the DNA sample was added to a 45 µl reaction volume of Master Mix in a 200 µl thin walled microfuge tube. Master Mix was 27 µl of ddH_2O, 5 µl of 0.1 mM of the primer *iss* pVAX1 Upper, 0.5 µl of 0.1 mM of the primer *iss* pVAX1 Lower, 0.25 [Amplitaq] AMPLITAQ (5 Units/ml)(Ampligene Kit, [Perkin-Elver] Perkin-Elmer, Foster City, CA), and 4 µl of 25 mM $MgCl_2$. A Perkin-Elmer PE2400 thermocycler was used to amplify *iss*. The amplification protocol used was 95°C for 5 minutes; 9 cycles of 95°C for 1 minute, 51.6°C for 30 seconds, and 72°C for 30 seconds; 25 cycles of 94°C for 30 seconds, 51.6°C for 30 seconds, and 72°C for 30 seconds; 72°C for 7 minutes; 4°C soak.

Please replace the paragraph beginning at **page 69, line 7**, with the following rewritten paragraph.

Using this procedure, a 400 base pair amplicon was generated. This amplicon was gel purified and removed from the low melt agarose using WIZARD PCR CLEAN-UP KIT [Wizard PCR Clean Up Kit] (Promega, Madison [Madiosn], WI). The *iss* amplicon was digested with Eco*RI* and Bam*HI* to prepare for cloning. At the same time, pVAX1, a plasmid specifically designed for use in DNA vaccines (Invitrogen, Carlsbad, CA) was also prepared for cloning. The digests were purified with WIZARD DNA CLEAN-UP KITS [Wizard DNA Clean UP Kits] (Promega) according to the manufacturer's recommendations. *iss* was ligated into pVAX1 in 1:1 and 3:1 ratios of molar ends (Sambrook et al. Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York (1989)) using T4 DNA ligase (GIBCO BRL,

Gaithersburg, MD). Ligations were transformed into chemically competent bacterial cells (XL-1 Blue, Stratagene, La Jolla, CA). Transformants were selected on kanamycin (50 µg/ml kanamycin, Amresco, Solon, OH). Kanamycin-resistant colonies were transferred to LB broth containing 50 µg/ml kanamycin. After overnight growth, some culture was reserved, and some was subjected to DNA isolation using WIZARD MINIPREPS [WizardMiniPreps] (Promega). DNA was restricted with Bam*HI* and Xba*I* (New England Biolabs, Beverly, MA). Desired clones were presumptively identified by the size of DNA fragments generated following plasmid restriction. The identity of the insert was confirmed by sequence analysis. The insert region was sequenced using IRD800 labeled T7 promoter/primer (TAATACGACTCACTATAGGG, SEQ ID NO:25) and BGH reverse primer (TAGAAGGCACAGTCGAGG, SEQ ID NO:26) (LI-COR, Lincoln, NE) with the SEQUITHERM [Sequitherm] EXCEL II (Epicentre, Madison, WI) cycle sequencing kit on a LI-COR 4000LS automated DNA sequencer. The *iss* vaccine construct can be seen in Figure 6.

In the Claims

For convenience, all pending claims are shown below.

30. (Amended) An isolated nucleic acid molecule comprising nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

31. (Amended) The isolated nucleic acid molecule of claim 30 further comprising nucleotides 1 to 33 of the nucleotide sequence SEQ ID NO:21, wherein the 33 nucleotides of the nucleotide sequence SEQ ID NO:21 are located 5' of nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

38. The immunogenic composition of claim 37 wherein the nucleic acid molecule comprises a vector.

39. The immunogenic composition of claim 38 wherein the vector is a plasmid.

40. The immunogenic composition of claim 38 wherein the vector is a viral vector.

41. The immunogenic composition of claim 37 wherein the nucleic acid molecule further comprises at least one regulatory sequence or control sequence operably linked to the nucleotide sequence encoding the polypeptide.

42. The immunogenic composition of claim 38 wherein the nucleic acid molecule further comprises an immunostimulatory sequence.

43. (Amended) The immunogenic composition of claim 38 wherein the nucleic acid molecule comprises nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22 or [a] an immunogenic subunit or immunogenic fragment thereof.

44. (Amended) The immunogenic composition of claim 38 wherein the nucleic acid molecule comprises nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

45. (Amended) The immunogenic composition of claim 43 wherein the nucleic acid molecule further comprising nucleotides 1 to 33 of the nucleotide sequence SEQ ID NO:21 located 5' of nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

46. A method for using an immunogenic composition comprising:

providing an immunogenic composition comprising a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an avian *E. coli* Iss polypeptide or an immunogenic fragment or subunit thereof; and

administering the immunogenic composition to a subject diagnosed with, at risk of, or exhibiting symptoms of an *E. coli* infection.

47. The method of claim 46 wherein the *E. coli* infection is selected from the group consisting of septicemic disease, colibacillosis, coligranuloma, peritonitis, salpingitis, synovitis, and omphalitis.

48. The method of claim 46 wherein the nucleic acid molecule comprises a vector.

49. The method of claim 48 wherein the vector is a plasmid.

50. The method of claim 48 wherein the vector is a viral vector.

51. The method of claim 46 wherein the nucleic acid molecule further comprises at least one regulatory sequence or control sequence operably linked to the nucleotide sequence encoding the polypeptide.

52. The method of claim 46 wherein the nucleic acid molecule further comprises an immunostimulatory sequence.

53. The method of claim 46 wherein the nucleic acid molecule comprises nucleotides 73 to 309 of SEQ ID NO:22 or a subunit or fragment thereof.

54. The method of claim 53 wherein the nucleic acid molecule comprises nucleotides 73 to 309 of SEQ ID NO:22.

55. The method of claim 53, the nucleic acid molecule further comprising nucleotides 1 to 33 of SEQ ID NO:21 located 5' of nucleotides 73 to 309 of SEQ ID NO:22.

56. The method of claim 46 wherein the subject is selected from the group consisting of a bird, a cow and a mink.

57. A method for making an immunogenic composition, the method comprising combining a pharmaceutically acceptable carrier and a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an avian *E. coli* Iss polypeptide or an immunogenic fragment or subunit thereof.

58. A method for vaccinating a subject comprising administering to the subject a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an *E. coli* Iss polypeptide or an immunogenic fragment or subunit thereof in an amount effective to result in an immune response that is specific for the Iss polypeptide.

59. The method of claim 58 wherein the nucleic acid molecule comprises a vector.

60. The method of claim 58 wherein the nucleic acid molecule further comprises a regulatory sequence or a control sequence operably linked to the nucleotide sequence encoding the polypeptide.

61. The method of claim 58 wherein the nucleic acid molecule further comprises an immunostimulatory sequence.

32. (Amended) The isolated nucleic acid molecule of claim 30 wherein nucleotides 73 to 309 of the [nucleic acid sequence] nucleotide sequence SEQ ID NO:22 is operably linked to a promoter functional in a host cell so as to form an expression vector.

33. (Amended) An expression vector comprising an isolated nucleic acid molecule comprising nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22, operably linked to [a] at least one regulatory sequence or control sequence.

35. (Amended) A method of using a nucleic acid molecule encoding an *E. coli* Iss polypeptide, the method comprising:

providing a host cell stably transformed with an expression vector comprising a nucleic acid molecule comprising nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22, operably linked to a least one regulatory sequence or control sequence ecognized by the host cell; and

expressing the nucleic acid molecule to yield an *E. coli* Iss polypeptide.

36. (Amended) The method of claim 35 wherein the nucleic acid molecule further comprises nucleotides 1 to 33 of the nucleotide sequence SEQ ID NO:21 located 5' of nucleotides 73 to 309 of the nucleotide sequence SEQ ID NO:22.

37. (Amended) An immunogenic composition comprising:

a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an avian *E. coli* Iss polypeptide or an immunogenic fragment or immunogenic subunit thereof; and

a pharmaceutically acceptable carrier.

62. The method of claim 58 wherein the subject is selected from the group consisting of a bird, a cow or a mink.

63. A method for treating or preventing disease in a subject caused by a complement resistant *E. coli* comprising administering to the subject a vaccine comprising a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising an *E. coli* Iss polypeptide or an immunogenic fragment or subunit thereof.

64. The method of claim 63 wherein the nucleic acid molecule comprises a vector.

65. The method of claim 63 wherein the nucleic acid molecule further comprises a regulatory sequence or a control sequence operably linked to the nucleotide sequence encoding the polypeptide.

66. The method of claim 65 wherein the nucleic acid molecule further comprises an immunostimulatory sequence.

67. (New) The immunogenic composition of claim 43 wherein the immunogenic composition generates an antibody response when administered to a subject.